CHAPTER 1

INTRODUCTION

Bacillus subtilis is a sporulating rod bacterium and is nonpathogenic to human beings (Zweers *et al.*, 2008), making it one of the most studied Gram-positive bacteria (Driks, 2002.). The ability of *B. subtilis* strains to produce a series of lipopeptides (surfactin, iturin and fengycin) has been documented for more than 60 years (Xiao *et al.*, 2008). Surfactin is a high-value bioproduct that offers advantageous application in various fields and was suggested as alternative compound to replace chemical surfactants. However, surfactin is an expensive lipopeptide, which makes it unable to compete effectively with chemical surfactants because the downstream process contributes up to 60% of its production cost (Oka *et al.*, 1993) due to the complexity of fermentation broth.

In recent years, a lot of effort has been expended in cutting down the downstream processing costs, including using foam fractionation (Davis *et al.*, 2001), acid precipitation (Chen *et al.*, 2007; Reis and Zydney, 2007), extraction using organic solvent, adsorption chromatography or a combination of these techniques. Unfortunately, all of these downstream procedure give low surfactin purity less than 65%, which makes them not good enough and leaves room for improvement in order to achieve higher performance on recovery and purity of surfactin final fraction. In addition, some of the approaches involving more than two-step treatment of fermentation broth make it impractical and less attractive for industry purposes (Lin and Jiang, 1997; Isa *et al.*, 2008). Besides, most of the conventional methods dealing with toxic organic solvents make the final product suffer from the loss of biosurfactant activity. Hence, there is a demand to develop more economic and environmentally friendly method to improve current downstream processing.

Surfactin separation efficiency from fermentation broth is the essential issue in developing commercial-scale processes. One of the alternative techniques for downstream processing is membrane filtration. Membrane filtration system was considered by previous researcher (Lin and Jiang, 1997; Isa *et al.*, 2007) for the purpose of recovery and purity of biosurfactants. Membrane filtration using pressure-

driven force applied to a membrane to dissolve and suspend species based on the size and molecular scale (Oka *et al.*, 1993) and widely used in various chemical and biochemical processes. More importantly, the membrane approach process involves no phase change (Mulligan and Gibbs, 1990), which enables the molecular structure to be preserved.

Membrane filtration meets downstream separation needs because the concentration and purification of the final product surpasses the limitations of traditional methods (Sen and Swaminathan, 2005; Chen *et al.*, 2008b; Reis and Zydney, 2007). The excellent characteristics of ultrafiltration (UF) include the minimized physical damage of biomolecules from shear effects, minimal denaturation, high recovery yield, and the avoidance of resolubilization. In this study, cross-flow UF equipped with polyethersulfone (PES) and hydrosart (HT) membrane with a molecular weight cut-off (MWCO) of 10 kDa and 30 kDa was used for the filtration of raw fermentation broth of *B. subtilis* MSH1and *B. subtilis* ATCC 21332. The final surfactin and protein concentration both in permeates and retentates were analysed to evaluate the performance of UF. This study aims to improve ultrafiltration (UF) technique to achieve a highly efficient and cost effective filtration process in a single-step UF technique for high recovery, purity and fully functional surfactin from fermentation broth that contain multicomponent compound.

The objectives for this research as follows:

1) To improve method of detection of surfactin and glucose using HPLC for fast detection and quantification of surfactin and glucose in complex fermentation broth
2) To investigate the fermentation process surfactin production by using *B. subtlis* MSH1 and *B. subtlis* ATCC 21332 strains in 5L bioreactor in terms of substrate utilization, biomass growth and surfactin concentration.
3) To determine most optimal variable of a single step UF technique for high recovery and purity of surfactin, and to evaluate the functionality of surfactin final fraction.